A real-life ImmunoCAT study: impact of molecular diagnosis through ImmunoCAP™ ISAC 112 on immunotherapy prescription in pollen-polysensitized patients in Catalonia, Spain


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Abstract

Background: Molecular diagnosis in allergology helps to identify multiple allergenic molecules simultaneously. The use of purified and/or recombinant allergens increases the accuracy of individual sensitization profiles in allergic patients.

Objective: To assess the impact of molecular diagnosis through the ImmunoCAP® ISAC 112 microarray on etiological diagnosis and specific immunotherapy (SIT) prescription. This was compared to the use of conventional diagnoses in pediatric, adolescent, and young adult patients with rhinitis or rhinoconjunctivitis and/or allergic asthma, sensitized to three or more pollen allergens of different botanical species.

Methods: A multicenter, prospective, observational study was conducted in patients aged 3-25 years who received care at the Allergology service of 14 hospitals in Catalonia from 2017 to 2020. Allergology diagnosis was established based on the patient's clinical assessment and the results of the skin prick test and specific immunoglobulin E assays. Subsequently, molecular diagnosis was conducted using ImmunoCAP® ISAC® 112 to recombinant and/or purified allergen components.

Results: A total of 109 patients were included; 35 (32.1%) were pediatric patients and 74 (67.9%) were adolescents or young adults (mean age: 18 years), with 58.0% being females. A change of 51.0% was observed in SIT prescription following molecular etiological diagnosis by means of a multi-parameter microarray.

Conclusions: Molecular diagnosis by means of multi-parameter tests increases the accuracy of etiological diagnosis and helps to define an accurate composition of SIT.

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Introduction

The first papers on specific immunotherapy (SIT) by Leonard Noon1 and Freeman were published in 1911, marking the beginning of the practical use of SIT in the treatment of allergic conditions. Several studies have been published in recent years, showing the beneficial effects of SIT, especially in respiratory conditions, rhinitis, and asthma.2 However, for SIT to be effective, the allergology diagnosis must be accurate and precise. For many years, immunoglobulin E (IgE)-mediated allergic reactions were diagnosed by means of heterogeneous protein combinations, both in vivo (skin prick test [SPT] or intradermal skin test) and in vitro (specific IgE [sIgE] assay against whole allergenic sources). These types of tests include complex protein combinations with varying numbers and amounts of allergens, which may lead to variable results for a single patient as well as to different degrees of correlation among the different techniques used.3

In recent years, allergology diagnostic techniques have advanced considerably with the advent of molecular diagnosis (MD), including the determination of isolated allergic molecules and/or the use of microarrays, such as ImmunoCAP® ISAC 112, allowing the determination of multiple molecules at the same time. The use of these purified and/or recombinant allergens, instead of whole allergenic sources, helps to increase the accuracy of individual sensitization profiles in allergic patients.4,5

Currently, several papers are published on the usefulness of incorporating purified and recombinant allergens to the usual clinical practice to establish a more accurate allergology diagnosis.6-8 An accurate diagnosis helps to differentiate cross-reactivity-related sensitizations—common to several species and, in general, without adequate response to SIT—from genuine sensitizations caused by a single species and, probably, candidates for initiating treatment with SIT.7 To date, no papers have been published that specifically assess whether molecular diagnosis through the commercially available ImmunoCAP® ISAC 112 microarray can result in changes in diagnosis and treatment of pediatric, adolescent, and young adult patients sensitized to three or more pollen species.

The objective of this paper was to assess the impact of molecular diagnosis through the commercially available ImmunoCAP® ISAC 112 microarray on etiological diagnosis and SIT prescription. This was compared to the use of conventional diagnoses, such as SPTs and/or whole extract sIgE assays in pediatric, adolescent, and young adult patients with rhinitis and/or allergic asthma, sensitized through SPT to three or more pollen allergens of different botanical species.

Methodology

Study design and patients

A multicenter, prospective, observational study was conducted between 2017 and 2020 at the Allergology Service of 14 hospitals in Catalonia involving patients aged 3-25 years, diagnosed with intermittent or persistent moderate or severe rhinitis or mild or moderate well-controlled asthma during last 2 years. For diagnosis, the criteria of the modified Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines and the Spanish Asthma Management Guidelines (GEMA), version 5.2, were followed.9,10

All the patients enrolled had to have positive SPTs to three or more pollens from different botanical species, must have resided in the same geographical region for at least last 2 years, and would have other allergic symptoms.
comorbidities, such as conjunctivitis and/or food allergies. Additionally, for the indication to start treatment with SIT, patients had to meet the following inclusion criteria: moderate or severe rhinoconjunctivitis (RC) or mild or moderate well-controlled asthma, a limited spectrum of clinically relevant allergies (maximum three allergies), inadequate response to usual pharmacotherapy (antihistamines, nasal corticosteroids, and/or asthma controllers), inadequate response to avoidance measures, confirmed sensitizations to specific allergens through SPTs and/or specific IgE blood tests, aimed at preventing progression from allergic rhinitis to asthma (the so-called “allergic march”), and preference to avoid long-term use of pharmacological treatments because of adverse effects, cost, or personal choice.

Exclusion criteria included patients with dermatological conditions preventing the performance of a SPT or blood draws; systemic inflammatory, autoimmune, or neoplastic disease; having received prior SIT against one or more allergens; and contraindications to SIT treatment against allergens (history of severe cardiovascular disease, concomitant treatment with beta-blockers, severe or uncontrolled asthma, psychiatric disorders, financial difficulties, or work or school problems).

The study was conducted in accordance with the Spanish Code of Medical Deontology, the Tokyo revision of the Declaration of Helsinki, and the International Ethical Guidelines for Health-Related Research Involving Humans; and in compliance with the requirements set forth in the “Ethics and Clinical Research” document of participating sites. Data collection and processing were conducted pursuant to Spanish Law 15/1999 on the protection of personal data, and all personal data and samples were assigned an independent code that was introduced into a database maintained using secure IT methods.

Prior to enrollment, the study was thoroughly explained to patients. All the patients (or their parents/legal guardians in the case of patients younger than 12 years) gave informed consent in writing to participate in the study.

**Study variables and diagnostic tests**

Allergology diagnosis was established based on the patient’s clinical assessment and the results of the SPTs and sIgE assays. SPT testing was conducted for the following allergens: *Parietaria judaica*, *Artemisia vulgaris*, *Salsola kali*, *Chenopodium album*, *Plantago lanceolata*, *Betula verrucosa*, *Corylus avellana*, *Platanus acerifolia*, *Cupressus arizonica*, *Olea europea*, *Phleum pratense*, profilin, and lipid transfer protein (LTP), with the technique described by Bousquet et al. and using histamine as a positive control and saline as a negative control.

Each investigator received molecular diagnosis results and, based on such data, established the patient’s clinical assessment, supplementary tests, and, according to the physician’s criteria and knowledge of molecular diagnosis, whether a change in etiological diagnosis and SIT prescription was required. All the data were captured in an electronic case report form, including demographical variables (age, gender, patient’s origin [urban, suburban, or rural area], and time of residence at their place of origin) and clinical variables (classification of asthma and rhinitis based on severity and seasonality; association with other allergic comorbidities, such as food allergy, atopic dermatitis, or urticaria/angioedema/anaphylaxis; family history of an atopic disease; toxic habits, both active and passive; and contact with domestic animals).

Subsequently, 20-mL peripheral venous blood sample was obtained using two Vacutest tubes with ice and coagulation activator. All samples collected were analyzed at the Phadia-Thermo Fisher applications laboratory at Hospital Universitari Vall Hebrón. The results obtained were determined using ISAC Standardized Units (ISU), and the manufacturer’s instructions were followed for determination thereof. Specific IgE were determined to whole allergenic sources with SPT positive results using the ImmunoCAP system (Phadia-Thermo Fisher, Vienna, Austria), and sIgE assays to recombinant and/or purified allergen components were determined with the commercially available ImmunoCAP ISAC 112 (Inmuno Solid-Phase Alergias Chip, Phadia-Thermo Fisher) microarray (Table 1).

**Statistical analysis**

Sample size of this study was calculated according to the following information: prevalence of respiratory conditions: 80% of pediatric, adolescent, and young adult patients in an outpatient allergology setting had some form of respiratory condition, such as rhinitis, rhinoconjunctivitis, and/or asthma; sensitization to allergens: within this group with respiratory conditions, 30-65% reported to have a positive SPT to three or more different allergens; and estimating sample size: based on the above data, the study aimed to capture a representative sample of patients who were suffering from respiratory conditions and had significant allergen sensitization (as evidenced by a positive SPT to three or more allergens). The calculations estimated that a minimum of 70 patients were required to ensure statistical accuracy. This data probably incorporated considerations beyond the scope of the study.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>List of 112 allergen components studied using the commercially available ImmunoCAP ISAC® 112 microarray (Inmuno Solid-Phase Alergias Chip, Phadia-Thermo Fisher).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Act d 1, Act d 2, Act d 8, Aln g 1, Alt a 1, Alt a 6, Amb a 1, Ana o 2, Ani s 1, Ani s 3, Api g 1, Api m 4, Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, Ara h 9, Art v 1, Art v 3, Asp f 1, Asp f 3, Asp f 6, Ber e 1, Bet v 1, Bet v 2, Bet v 4, Bla g 5, Bla g 7, Blo t 5, Bos d 4, Bos d 5, Bos d 6, Bos d 8, Bos d lactoferrin, Can f 1, Can f 2, Can f 3, Can f 5, Che a 1, Cla h 8, Cor a 1.0101, Cor a 1.0401, Cor a 8, Cor a 9, Cry c 1, Cup a 1, Cyn d 1, Der f 1, Der f 2, Der p 2, Der p 10, Equ c 1, Equ c 3, Fel d 1, Fel d 2, Fel d 4, Gad c 1, Gal d 1, Gal d 2, Gal d 3, Gal d 5, Gly m 4, Gly m 5, Gly m 6, Hev b 3, Hev b 5, Hev b 6.01, Hev b 8, Jug r 1, Jug r 2, Jug r 3, Lep d 2, Mal d 1, Mer a 1, Mus m 1, Ole e 1, Ole e 7, Ole e 9, Par j 2, Pen m 1, Pen m 2, Pen m 4, Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, Phl p 12, Pla a 1, Pla a 2, Pla a 3, Pla a 1, Pol d 5, Pru p 1, Pru p 3, Sal k 1, Sesi 1, Tri a 14, Tri a 19.0101, Tri a 2A_Ti, Ves v5</td>
<td></td>
</tr>
</tbody>
</table>

**ImmunoCAT study**

A real-life ImmunoCAT study was conducted to test the accuracy of the ImmunoCAP ISAC 112 microarray (Inmuno Solid-Phase Alergias Chip, Phadia-Thermo Fisher). Each investigator received molecular diagnosis results and, based on such data, established the patient’s clinical assessment, supplementary tests, and, according to the physician’s criteria and knowledge of molecular diagnosis, whether a change in etiological diagnosis and SIT prescription was required. All the data were captured in an electronic case report form, including demographical variables (age, gender, patient’s origin [urban, suburban, or rural area], and time of residence at their place of origin) and clinical variables (classification of asthma and rhinitis based on severity and seasonality; association with other allergic comorbidities, such as food allergy, atopic dermatitis, or urticaria/angioedema/anaphylaxis; family history of an atopic disease; toxic habits, both active and passive; and contact with domestic animals).
such as the expected effect size (the magnitude of difference or relationship expected), power of the study (the probability of detecting an effect if there is one, set at 80% or 0.8), and the level of significance (the probability threshold for accepting the observed effect as real, often set at 5% or 0.05).

Statistical accuracy: Achieving enough statistical accuracy means the study was powered to discover a true effect or difference with high probability, minimizing the risk of type I (false positive) and type II (false negative) errors. The sample size of 70 patients was sufficient to meet these statistical requirements, given the specific context of the study—namely, the prevalence of respiratory conditions and allergen sensitization in the patient population.

The SPSS statistical package, version 23.0, was used for the statistical analysis. A descriptive analysis was conducted to gain insight into the distribution of frequencies of variables. Two patient groups were defined based on their age: pediatric patients (<11 years old) and adolescent and young adult patients (11–25 years old). Ratios between study variables were analyzed using Student’s t-test and ANOVA test. The results obtained from the ImmunoCAP™ ISAC 112 multi-parametric test were presented using box plots by component, age group, and type of SIT based on allergens prior to and after the application of molecular diagnosis, expressed as proportions and by age group. The statistical significance level was set at $P < 0.05$.

Results

Characteristics of study patients

Of the 109 patients included in the study, 35 (32.1%) were pediatric patients and 74 (67.9%) were adolescents or young adults, with a mean age of 18 years, with 58% females (Table 2).

Rhinconjunctivitis was diagnosed in 94.5% ($n = 103$) of the patients, while 45.9% ($n = 50$) were diagnosed with asthma and 48.6% ($n = 53$) had some type of food allergy, mostly corresponding to nuts (32.1% [$n = 35$]) and fruit (27.5% [$n = 30$]) (Table 3) and because of LTP sensitization.

**Table 2** Demographic data of the study population.

<table>
<thead>
<tr>
<th>Age at inclusion (years, mean [min, max])</th>
<th>18.2 (3, 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups (years, n [%])</td>
<td></td>
</tr>
<tr>
<td>&lt;11</td>
<td>35 (32.1)</td>
</tr>
<tr>
<td>11-25</td>
<td>74 (67.9)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>58 (53.2)</td>
</tr>
<tr>
<td>Male</td>
<td>51 (46.8)</td>
</tr>
<tr>
<td>City of origin, n (%)</td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>5 (4.6)</td>
</tr>
<tr>
<td>Suburban</td>
<td>12 (11.0)</td>
</tr>
<tr>
<td>Urban</td>
<td>92 (84.4)</td>
</tr>
<tr>
<td>Years living in the same area, mean (min, max)</td>
<td>13 (2, 25)</td>
</tr>
</tbody>
</table>

N = 109 (100%).

**Table 3** Clinical data, n (%), of the study population ($N = 109$) based on the patient age group.

<table>
<thead>
<tr>
<th></th>
<th>&lt;11 years (n = 35)</th>
<th>11–25 years (n = 74)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaphylaxis</td>
<td>0 (0.0)</td>
<td>6 (8.1)</td>
</tr>
<tr>
<td>Asthma</td>
<td>21 (60.0)</td>
<td>29 (39.2)</td>
</tr>
<tr>
<td>Severity of asthma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>15 (75.0)</td>
<td>22 (78.6)</td>
</tr>
<tr>
<td>Moderate</td>
<td>5 (25.0)</td>
<td>6 (21.4)</td>
</tr>
<tr>
<td>Severe</td>
<td>0 (0.0)</td>
<td>3 (4.2)</td>
</tr>
<tr>
<td>Rhinoconjunctivitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>12 (38.7)</td>
<td>28 (39.4)</td>
</tr>
<tr>
<td>Moderate</td>
<td>19 (61.3)</td>
<td>40 (56.3)</td>
</tr>
<tr>
<td>Severe</td>
<td>0 (0.0)</td>
<td>3 (4.2)</td>
</tr>
<tr>
<td>Urticaria/angioedema</td>
<td>4 (11.4)</td>
<td>14 (18.9)</td>
</tr>
<tr>
<td>Food allergy</td>
<td>14 (40.0)</td>
<td>39 (52.7)</td>
</tr>
<tr>
<td>Nuts</td>
<td>11 (31.4)</td>
<td>24 (32.4)</td>
</tr>
<tr>
<td>Fruit</td>
<td>7 (20.0)</td>
<td>23 (31.1)</td>
</tr>
<tr>
<td>Egg</td>
<td>6 (17.1)</td>
<td>-</td>
</tr>
<tr>
<td>Fish</td>
<td>4 (11.4)</td>
<td>-</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>4 (11.4)</td>
<td>6 (8.1)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>-</td>
<td>6 (8.1)</td>
</tr>
<tr>
<td>Atopy familiar background</td>
<td>29 (82.9)</td>
<td>49 (66.2)</td>
</tr>
<tr>
<td>Toxic habits</td>
<td>0 (0.0)</td>
<td>2 (2.7)</td>
</tr>
<tr>
<td>Passive toxic habits</td>
<td>5 (14.3)</td>
<td>3 (4.0)</td>
</tr>
<tr>
<td>Furry animals</td>
<td>8 (22.9)</td>
<td>23 (31.0)</td>
</tr>
</tbody>
</table>

Allergen sensitizations based on SPT results and determination of specific IgE to whole extracts

Figure 1 shows the sensitizations diagnosed by means of SPT. The most common sensitizations to pollen for both age groups were to *O. europaea* and grass, followed by *P. acerifolia* and *A. vulgaris*, for pediatric patients, and *C. arizónica*, *P. acerifolia*, and *P. judaica* for adolescents and young adults. Concerning non-pollen-related allergens, sensitization to domestic dust mites, fungal spores of *A. alternata*, and animal epithelium (cats and dogs) stood out in both groups. Sensitization to LTP was more prevalent than sensitization to profilin protein, also for both groups.

Figure 2 shows the relationship between SPT positivity and sIgE assay to the whole extract of the inhalant allergens studied.

Changes in etiological diagnosis following the application of MD ImmunoCAP™ ISAC 112

In the pediatric patient group, the most prevalent sensitizations were those to olive molecule Ole e 1, followed by those to grass molecules Phl p 1, Phl p 4, and Cyn d 1; the predominant allergen of the fungal spores of *A. alternata* Alt a 1; *Cupressaceae* molecules Cup a 1 and Cry j 1; domestic dust mite molecules Der p 2, Der f 2, Der p 1, and Der f 1; cat Fel d 1; and London plane tree Pla a 1, Pla a 2, and Pla a 3. However, in contrast to the pediatric patient group, among adolescent and young adult patients, the third most
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Figure 1 Sensitization to inhalant allergens based on skin prick test by age.

Figure 2 Skin prick test positivity and determination of whole extract specific IgE to the inhalant allergens studied. LTP: lipid transfer protein; SPT: skin prick test; sIgE: specific IgE.

prevalent sensitization was to Cupressaceae pollen Cup a 1 and Cry j 1, followed by the parietaria pollen marker molecule Par j 2, London plane-tree molecule Pla a 2, and the molecules of non-pollen-related allergens Der p 2, Der f 2, Der p 1, Der f 1 (domestic dust mites), Fel d 2 (cat), and Alt a 1 (A. alternata). Molecular diagnosis results using the commercially available microarray are shown in Figure 3.

Changes in SIT prescription following application of MD ImmunoCAP™ ISAC 112

Figure 4 shows the main changes observed in SIT prescription. The pediatric patient group showed an increase in SIT prescription for pollens of O. europaea (20.0%), C. arizonica (8.3%), and grass (5.7%). In the adolescent and young adult group, the change was not noticeable. However, it did show an increase to the expense of C. arizonica (13.5%) and a statistically significant decrease in the prescription of SIT for P. acerifolia (4.0%) and A. vulgaris (2.7%) pollens. Concerning non-pollen-related allergens, a significant increase was observed in both age groups, mainly for the spore of A. alternata fungus in the pediatric patient group (5.7%) and for domestic dust mites (O. pteronyssinus and/or D. farinae) in the adolescent and young adult patient group (6.8%).

Similar results were obtained for SIT prescription based on the results of different tests for inhalant allergens corresponding to the pollens of Olea europaea and grass, domestic dust mites, or spores of Alternaria alternata fungus. However, other comparisons between SPT, sIgE, and ISAC® 112 showed low levels of agreement, such as SPT to the Cupressaceae family and ISAC® 112 to Cup a 1 and Cry j 1 molecules, or in the case of pediatric patients, SPT to
dog dander and cat dander and ISAC® 112 to Fel d 1, Can f 1, Can f 2, Can f 3, or Can f 5 for non-pollen-based inhalant allergens.

Because of these changes, global SIT prescription varied in 51.0% of the patients studied. Prior to using molecular diagnosis, SIT was prescribed in 48.6% of pediatric patients and 41.9% of adolescent and young adult patients. Following the application of molecular diagnosis, this prescription experienced a statistically significant increase in both groups, to 68.6% of pediatric patients and 58.1% of adolescents and young adults. In the pediatric patient group, a 29.0% increase was observed in patients who were not prescribed SIT previously and a 9.0% decrease in those who were prescribed SIT previously. Among patients with prescribed SIT, 13.0% maintained the treatment but with a change in the number and/or type of inhalant allergens prescribed. In the adolescent and young adult group, changes to SIT prescription were similar, with a 24.0% increase in the number of patients with no SIT prescription with a conventional diagnosis, an 8% decline in patients with previously prescribed SIT, and a change in the number and/or type of inhalant allergens prescribed in 19.0% of the patients who were prescribed SIT previously.

Discussion

The results of this multicenter, prospective, observational study in pediatric, adolescent, and young adult patients showed that, following molecular etiological diagnosis by means of the multi-parameter ImmunoCAP™ ISAC 112 microarray, global SIT prescription changed in 51% of the cases. The sub-analysis by age group showed that, after molecular diagnosis, the number of SITs prescribed in patients who were not prescribed SIT previously rose by 29% in the pediatric group and by 24% in the adolescent and young adult group. In both age groups, the most common sensitizations with the SPT technique were those to *Olea europaea* and grass. Following the determination of whole extract sIgE, the highest percentage of patients with negative SPTs and positive for sIgE were obtained with *Platanus acerifolia, Cupressus arizonica, Parietaria judaica,*
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These results aligned with the results published by Depreux et al., in which the authors found that, after molecular diagnosis using ImmunoCAP™ ISAC 112 (n = 42), the indications for SIT and compositions of the prescribed vaccines were changed in 59% of cases. However, these results cannot be directly compared to our results because their study included both pediatric and adult patients (age range 7–70 years), and some of the patients were sensitized to only two pollens.

Other studies also assessed changes in SIT prescription following molecular diagnosis but did not use multi-parametric tests, which could lead to diagnostic bias. In the pediatric population, we found two Italian studies comprising 462 and 651 patients with food or respiratory allergies, where molecular diagnosis was used to analyze inhalant allergens with SPT-positive results (12 and 8 allergenic molecules, respectively), and where a change was observed in SIT prescription of 50% and 47%, respectively. In our geographical region, we also found studies in the pediatric population with similar results, but they also limited molecular assessment to the results of SPT.

Among the studies conducted in the population aged 5–65 years using molecular diagnosis with few molecules, we found a study conducted comprising 1263 patients with a clinical history of allergic and *Olea europaea* (**Figure 2**). Following molecular diagnosis, the most relevant differences in both groups were observed for *Olea europaea* and grass, *Cupressus arizonica*, and *Parietaria judaica*. An increase in SIT prescription was observed for the pollens of *Olea europaea*, *Cupressus arizonica*, and grass in pediatric patients, and with pollens of *Cupressus arizonica* in adolescents and young adults. In the second group, SIT prescription decreased for *Platanus acerifolia* and *Artemisia vulgaris*.

Regarding other studies assessing changes in SIT prescription following molecular diagnosis using multi-parametric tests, we did not discover any published studies exclusively in the pediatric and adolescent/young adult populations.

However, we found three studies that used multi-parametric test and showed some similarity to our study. The first study comprised 300 patients aged 3–82 years, sensitized to three or more pollen aeroallergens from different species, where molecular diagnosis was conducted using ImmunoCAP™ ISAC 112. The second study conducted comprised 141 patients with a mean age of 31 ± 13.63 years, diagnosed with rhinoconjunctivitis and/or allergic asthma, where the ISAC® 96 test was used for molecular diagnosis. Both studies identified that the most prevalent sensitizations to pollen with SPT were to *Olea europaea* and grasses. Following molecular diagnosis, SIT prescription changed in 51% of the cases in the study using ImmunoCAP™ ISAC 112, with increased prescription in 26% cases. In the study analyzing 96 molecules, the change in SIT prescription was 54%. Additionally, in the second study, few matches were found between SPT and ISAC® 96 test for molecules, such as Pla a, Pla a 2, and Phl p 5, highlighting the importance of using multi-parametric tests based on molecular diagnosis.

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In one of the studies, assessing grass and olive molecules in 281 patients, a change in the composition of SIT prescription was observed after molecular diagnosis in 52.9% of the patients. Another study, conducted among 70 children, showed that SIT prescription following molecular diagnosis was modified in 54.3% of the cases, with a 33% increase in SIT prescription against a single allergen, and a decrease in SIT prescription in 9.3% of pediatric patients and 8% of adolescent and young adult patients. Among the studies conducted in the population aged 5–65 years using molecular diagnosis with few molecules, we found a study conducted comprising 1263 patients with a clinical history of allergic

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**Figure 4** Changes in SIT prescription based on inhalant allergens. Statistically significant.
rhinitis related to grass and olive pollen SPT sensitizations, where a 56.8% change in SIT prescription was observed following molecular diagnosis.18

Using SPT results to determine molecules to analyze through molecular diagnosis may rule out a correct diagnosis in a considerable number of symptomatic patients whose sensitizations have not been tested using this assay. This diagnostic bias results from the limitations of SPT in case of including complex protein combinations with allergenic and non-allergenic material, a variable number of allergens, and from the variability dependent on the operator conducting the test.6,20,21 All this could lead to variable results in a single patient and to different degrees of correlation among the different techniques used.5

These facts could explain the proportion, although expectedly small of patients with negative SPT but positive sIgE, which indeed exhibited only very low levels of positive sIgE, suggesting a subtler form of sensitization in our population. Other factors that could explain these differences were false-positive sIgE levels because of elevated total IgE levels, as observed in conditions such as eczema, and the well-known variability of some allergens in the extracts used, which could significantly affect the sensitivity and specificity of SPT. This, along with the poor quality of some allergen extracts used in testing, could lead to false-negative results. In that sense, it is important to highlight the availability and efficacy of allergen extracts in Catalonia, Spain. A wide availability of allergen extracts was noticed, as described in the “Methods” section. However, the efficacy of most of these extracts was not supported by clinical trials but only by real-world evidence. In the current study, the potential for skin prick test results to be contaminated by antihistamines, which could suppress skin reactivity, was doubtful. This was probably because all patients were tested with histamine as a positive control. If histamine was not positive, then patients were not included in the study.

In complex aeroallergen sensitization areas, especially in the absence of data on prevalent sensitizations, the use of molecular diagnosis versus tests, such as SPT and sIgE, using commercially available extracts may change SIT prescription in approximately 50% of the cases.22 It was shown that these changes in immunotherapy prescription after using molecular diagnosis could be useful.7,15,21 For example, if more than 12 or 13 sIgEs were to be detected, then the multiple assay was more profitable than the simple diagnostic method.24 Additionally, some grass or olive pollen allergen sensitization patterns could identify patients at higher risk of suffering adverse reactions during immunotherapy.25

**Strengths and Limitations**

The main limitation of the study was the heterogeneity in selecting the prescribed SIT because of the absence of a specific protocol for the study. This may lead to a lack of generalization of results. However, participating investigators had wide expertise and knowledge in the field of etiological diagnosis. One of the strengths of the study, based on our knowledge to date, was that this was the first multicenter study with the characteristics conducted in the autonomous region of Catalonia among an exclusively pediatric, adolescent, and young adult population.

**Conclusions**

Molecular diagnosis using the commercially available ImmunoCAP™ ISAC 112 microarray offers an accurate etiological diagnosis. Its use can change SIT prescription in more than half of patients previously tested by SPT or whole extract sIgE assays. These changes allow for a more accurate determination of correct SIT composition.

**Author Contributions**

Manuscript concept and design: M.S-M, M.L-H, T.G-B; Statistical analysis and interpretation of data: CL, T.G-B, M.L-H, M.S-M M.T.; Drafting of the original manuscript: T.G-B. Reviewed and edited the manuscript: All authors provided critical review of the manuscript and approved the final version. Obtained funding: T.G-B, M.S-M.

**Conflict of Interest**

M.L-H report research funding from Thermofisher. None of the other authors have anything to disclose.

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**References**


